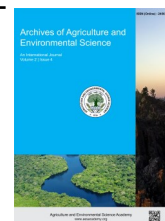




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ORIGINAL RESEARCH ARTICLE



Biological control of eucalyptus Gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) in Punjab, India

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*Leptocybe invasa**Megastimus viggianii**Quadrastichus mendeli***ABSTRACT**

In this investigation, biological control of eucalyptus Gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) in Punjab, India was investigated. The genus *Leptocybe* Fisher & La Salle (Hymenoptera: Eulophidae) was founded with its type species, *L. invasa* from Israel in 2004. Adult wasps of *L. invasa* induce galls on the shoots and midribs of leaves of *Eucalyptus* sp. and also damage nursery seedlings and young plantations. In the present study, survey was carried out from the year 2012 to 2014, on gall wasp infestation in different eucalyptus growing areas of Punjab. Observations revealed that eucalyptus growing areas in Punjab were under the threat of attack by gall wasp, *L. invasa*. During the present study, biological control agents *Megastimus viggianii* and *Quadrastichus mendeli* have been successfully applied in the gall infested areas of Punjab, from 2012 to 2017, where infestation of gall wasp was regularly decreased and came below 10 % by July, 2017. Consequently, *Eucalyptus* gall forming insect *L. invasa* has been controlled successfully by applying the biological control through releasing the parasitoids (*Megastimus viggianii* and *Quadrastichus mendeli*), in Punjab, India.

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INTRODUCTION

Leptocybe invasa Fisher & La Salle (Hymenoptera: Eulophidae) is the most serious insect pest of Eucalyptus. Its outbreak has been observed since 2002 in the Mediterranean region (Mendel *et al.*, 2004). This gall wasp has been making considerable damage in the *Eucalyptus* nurseries and plantations. This insect reproduces parthenogenically (Mendel *et al.*, 2004). Conventional control measure like sweep net collection, sticky traps, colour and light trap and pesticides could not be successful due to its concealed nature (Jacob and Siva Kumar, 2012). In its native place, Australia, this insect and its damage were so negligible that it was never noticed. Within a couple of years, *Eucalyptus* plantations and nurseries throughout the tropical and subtropical countries came under the attack of this wasp (Mendel *et al.*, 2004; Philip *et al.*, 2009; Karunaratne *et al.*, 2010) and brought havoc in *Eucalyptus* plantations and ultimately exerted a pressure on plantation industry. Meanwhile variations in the

incidence and intensity of gall in clones and seed origin plants were observed by various researchers (Jacob and Kumar, 2009; Thu *et al.*, 2009). In India, distribution of different parasitoids like *Megastigmus viggianii* (Viggiani *et al.*, 2000; Ankita and Poorani, 2008; Yousuf *et al.*, 2017); *Aprostocetus* sp. & *Parallelaptera* sp. (Kumari, 2009; Vastrad *et al.*, 2010); *M. dharwadicus* (Narendran *et al.*, 2010; Verghese *et al.*, 2013; Ramanagouda and Vastrad, 2015); *Quadrastichus mendeli* (Shylesha, 2008; Jacob *et al.*, 2015) was reported on gall wasp *L. invasa*. Classical biological control was also reported successful in Israel (Kim *et al.*, 2008). Survey was carried out from 2012 to 2014 of gall wasp infestation in different Eucalyptus growing areas of Uttarakhand, Uttar Pradesh, Haryana, Punjab (Yousuf *et al.*, 2017), observations revealed that in Punjab, Eucalyptus growing areas were under threat of attack by gall wasp, *L. invasa*. During study biological control agents like *Megastigmus viggianii* and *Q. mendeli*, mainly dominating (90%) parasitoids *M. viggianii* have been applied in different gall infested areas (districts) of Punjab,

from 2012 to 2017, where infestation of gall wasp was regularly decreased and came below 10 % by July 2017.

MATERIALS AND METHODS

Parasitoids procurements

Eucalyptus twigs containing parasitized galls with parasitoid *Megastimus viggianii* and *Quadrastichus mendeli* packed in four cartons (Boxes) were received from Dr. A. N. Shylesha, National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, on 13th October 2012 and these were brought to FRI, Dehra Dun on 15th October 2012 for their mass multiplication and application in biological control of Eucalyptus gall wasp *L. invasa*. Second time more culture of parasitoid dominating *M. viggianii* was taken from NBAIR on 08 November, 2012 and these parasitoids were multiplied at Forest Protection Division (Forest Entomology Discipline), FRI in fresh green galls of Eucalyptus seedlings, kept in double meshed outdoor cages and also mass multiplied in multiplication chamber at Satyal nursery, Hoshiarpur, Punjab, India (Figure 1).

Extraction method of parasitoids

For extraction of parasitoids from the matured parasitised galls, which were red in colour, having the mature parasitoids were kept for emergence of parasitoids. Parasitoids were collected with the help of sleeves and their purification was done with the help of aspirator. Plants with mature galls having parasitoids

were kept under the nylon/ muslin cloth sleeves, with glass tubes tightened with rubber band at the top of sleeves, so that the emerged parasitoids were collected in glass tubes (Figure 4).

Purification of parasitoids

It has been observed that during the extraction of parasitoids in sleeves, both parasitoids and gall wasp were emerged and congregated in glass tubes. From glass tube collection, parasitoids and gall wasps were separated with the help of aspirator. After taking the pure culture, gall wasp *L. invasa* was provided 50% water diluted honey as food, and these gall wasps were introduced into the multiplication chamber with fresh quality seedlings, so that after infestation green galls could be produced. Another lot having parasitoids, was also provided 50% water diluted honey, as food (Figure 2) and those parasitoids were released in gall infested Eucalyptus nurseries or plantations in fields in Punjab.

Statistical analysis

Normality testing, Kolmogorov-Smirnov and Shapiro-Wilk test were conducted with the null hypothesis "Infestation intensity is not normal". The probability significance for both the years (Oct -Nov, 2015 and May-Jul, 2017) was also carried out (Figure 6).

Formula used for Gall intensity (%)

Gall intensity (%) = Total number of leaves/ total number of gall infested leaves*100

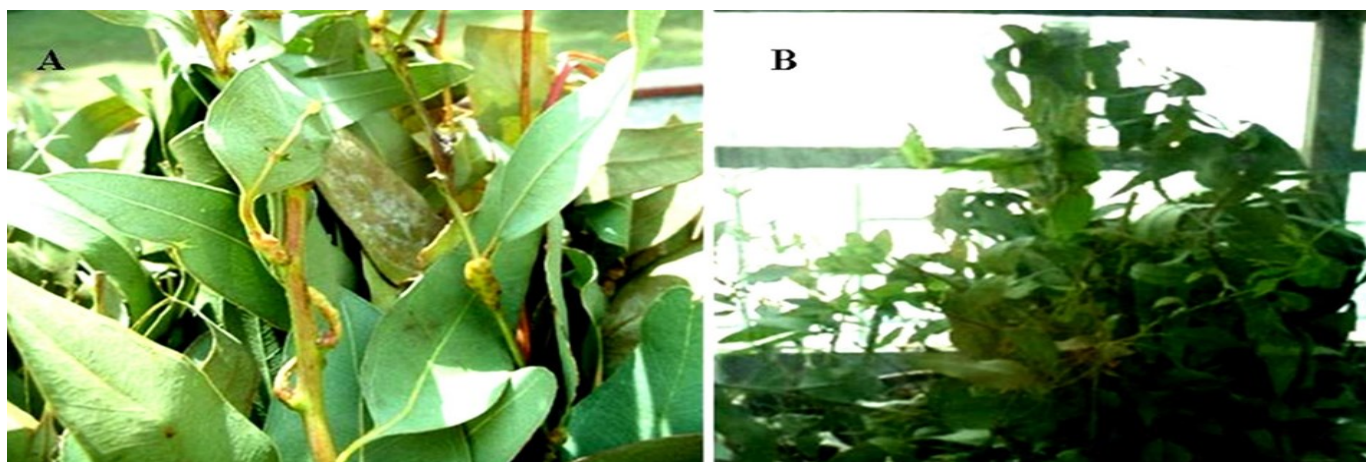


Figure 1. (A-B): A, Eucalyptus twigs with parasitoids (*Megastimus viggianii* and *Quadrastichus mendeli*), from NBAIR, Bengaluru; B, laboratory parasitisation in fresh galls at Forest Protection Division, FRI, Dehradun.



Figure 2. Parasitoids in test tubes with food (Honey solution 50% water solution).

RESULTS AND DISCUSSION

Release of parasitoids in Nurseries and plantations

During 2013, for the experimental release, Bhunga (release site) and Kailon (control site), Dasuya road Hoshiarpur (Punjab) were selected and after release of parasitoids; observations were regularly taken on the effect of release of parasitoids, having taken the basis of the data on gall intensity in the experimental sites.

Non experimental release of parasitoids was also carried out at twelve (12) other sites in Hoshiarpur, Pathankot and Gurdaspur for controlling the gall wasp infestation in Punjab in 2013. Further in 2014, for release of parasitoids, 35 new sites were selected in Jalandhar (Phillaur), Kapurthala (Phagwara), Ludhiana, Gurdaspur, Rupnagar (= Ropar) and Hoshiarpur. On all these selected sites, parasitoids were released in Eucalyptus nurseries and plantations. After 2014 decline of the Eucalyptus gall infestation was started, therefore in 2015-16 and 2016-17 only limited releases were carried out which were essentially required. On the basis of the gall infested sites during this period 5 new sites for release of parasitoids in Hoshiarpur, Patiala, Jalandhar, Pathankot and Ludhiana were taken and the parasitoids were released for biological control of Eucalyptus gall wasp. Details of release of parasitoids in different years/ time periods and the sites where parasitoids were released are given below.

Release of parasitoids during 2013

Details of the release carried out during the year 2013 are given below (Table 1).

Release of parasitoids during the year 2014

During the year 2014, release of parasitoids was started from April 2014. As a whole approximately 55,100 parasitoids were released at 42 localities, suffering from Eucalyptus gall wasp infestation in various districts of Punjab, including Hoshiarpur, Kapurthala, Ludhiana, Gurdaspur, Ropar (Rupnagar) and Jalandhar. Most of the releases were concentrated at district Hoshiarpur and nearby areas, where several localities, including nurseries, village areas and road side Eucalyptus plantations were suffering from serious gall infestation. These areas were targeted for the release of parasitoids for controlling the infestation of gall wasp. Release was continuous from April to May, 2014, after winter season, it was the first annual release season of parasitoids. Second time emergence of parasitoids and their release were continued from September to October, 2014. During November emergence is very limited and in December, January February, emergence was almost nil. In last week of March, very less number of Parasitoids was emerged. Good emergence of parasitoids was observed in April-May and September-October. Details of releases are given below (Table 2).

Table 1. Release of parasitoids in Punjab during the year 2013.

| S.N. | Date of release | Locality | No. of Parasitoids (Approx.) released |
|------|-----------------|--|---------------------------------------|
| 1. | 04.04.2013 | Bhunga, Dasuya Road, Hoshiarpur | 6000 |
| | | Satyal Nursery, Hoshiarpur | 4500 |
| 2. | 04.09.2013 | Bhunga, Dasuya Road, Hoshiarpur | 3500 |
| 3. | 05.09.2013 | Satyal Nursery, Hoshiarpur | 3000 |
| 4. | 01.10.2013 | Nursery near Satyal, Hoshiarpur | 3300 |
| 5. | 02.10.2013 | NangalShahidan on Chandigarh Road, Hoshiarpur | 3500 |
| 6. | 16.10.2013 | Randhawa village on Dasuya Road, Hoshiarpur | 3000 |
| | | Munkhurd, Hoshiarpur | 3500 |
| | | Salempur, Hoshiarpur | 2500 |
| 7. | 17.10.2013 | Railway crossing, Gurdaspur Road, and Mukerian, Hoshiarpur | 3500 |
| | | Musahibpur, near Mukerian, Hoshiarpur | 4000 |
| | | Malikpur Chowk, Pathankot | 3000 |
| 8. | 18.10.2013 | Ibrahimpurvillage, near Garh Shankar | 3500 |
| | | Garhhazpurvillage, near Garh Shankar | 4000 |
| 9. | 29.10.2013 | Chintapurni Mata Mandir, Road, Bahadurpur, Hoshiarpur | 2500 |
| 10. | 30.10.2013 | Bassi Janna, Hoshiarpur | 2000 |
| 11. | 31.10.2013 | Bhunga, Dasuya Road, Hoshiarpur | 2500 |
| | | Total | 57,800 |



Figure 3. (A-J). Mass multiplication of gall wasp, *Leptocybe invasa* (A, Multiplication chamber; B, galls on petioles; C, gall multiplication on *Eucalyptus* plants; D, close up of twigs with galls; E, gall in close up; F, mature galls; G, larval stage; H, pupal stage; I, emergence hole; J, newly emerged adult *Leptocybe invasa* ♀).

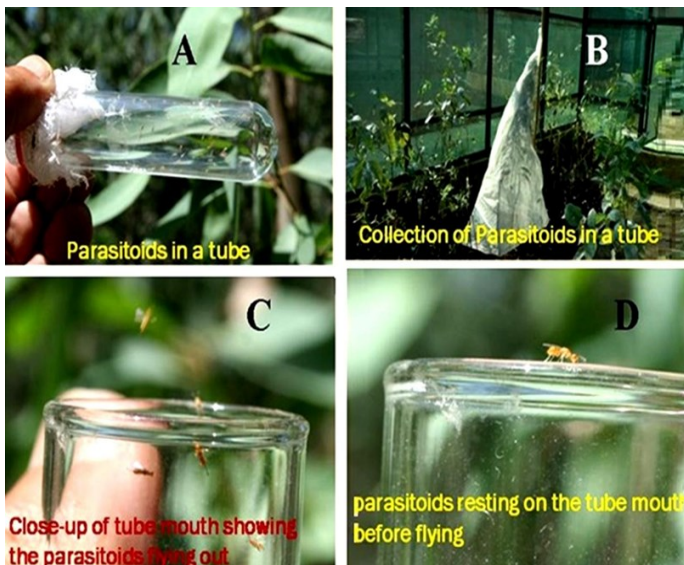


Figure 4 (A-D). A, parasitoids taken in glass tube; B, Collection of parasitoids; C, parasitoids in tube; D, Parasitoid in close up.



Figure 5 (A-D). Parasitoids release sites, Hoshiarpur: A, Bahadurpur; B, Dasuya road; C, Satyal nursery; D, Near Satyal nursery.

Table 2. Release of parasitoids in Punjab during the year 2014.

| S.N. | Date of release | Localities | Number of Parasitoids (Approx.) released |
|------|-----------------|--|--|
| 1. | 23.04.2014 | Phillaur, Jalandhar | 3000 |
| 2. | 24.04.2014 | Dasuya-Mukerian, road side and Mahilpur, Hoshiarpur | 2000 |
| 3. | 30.04.2014 | Phularia near Soma company office, Phagwara, Jalandhar | 1500 |
| 4. | 30.04.2014 | Omex-resort on GT road, Ludhiana | 900 |
| 5. | 01.05.2014 | Pattan, on the bank of Ravi river, Gurdaspur | 1200 |
| 6. | 01.05.2014 | Galladi village in Gurdaspur | 1100 |
| 7. | 09.09.2014 | Mangal village on Anandpur sahib road, Ropar | 1200 |
| 8. | 10.09.2014 | Pojewalaon, Anandpur sahib road, Ropar | 1500 |
| | | Sailakhurd near Garhshanker, Hoshiarpur | 1100 |
| | | Banjar bag, Hoshiarpur | 2000 |
| | | Chidal, Hoshiarpur | 1000 |
| | | Islamabad, Hoshiarpur. | 1000 |
| 9. | 11.09.2014 | PangeonPhagwara road, Hoshiarpur | 1500 |
| | | TuttoMajra near Mahilpur, Hoshiarpur | 1000 |
| | | Bhagbai, Garh Shankar | 2000 |
| | | Roorkee kala, Hoshiarpur | 1000 |
| 10. | 23.09.2014 | Sangatpur, Ropar | 1500 |
| | | Gara on Anandpur sahib road, Ropar | 1500 |
| 11. | 24.09.2014 | Bassi Mustafa, Una road, Hoshiarpur | 1400 |
| | | Dada Merha, Hoshiarpur | 2000 |
| | | Kharka, Hoshiarpur | 1500 |
| | | Patialia , Hoshiarpur | 1500 |
| | | Jahan Kalan, Hoshiarpur | 1200 |
| | | Chakguzra, Jalandhar road, Hoshiarpur | 1500 |
| | | Kahrisahri, Hoshiarpur | 1000 |
| | | Ram Nagar, Hoshiarpur | 1500 |
| | | Bhootgarh on Chandigarh road, Hoshiarpur | 1200 |
| | | Bhikhowal, Hoshiarpur | 1500 |
| 12. | 25.09.2014 | Khurdan on Dasuya road, Hoshiarpur | 1000 |
| | | Patheh on Jalandhar road, Hoshiarpur | 1500 |
| 13. | 14.10.2014 | TuttoMajra on Chandigarh Road, Hoshiarpur | 1000 |
| 14. | 15.10.2014 | Nasrula on Jalandhar road, Hoshiarpur | 1100 |
| 15. | 16.10.2014 | Rangriwaal, Jalandhar | 1000 |
| 16. | 28.10.2014 | Chuharwali, Jalandhar | 2000 |
| | | Gondpur, Hoshiarpur | 1000 |
| | | Road side plantation at Saraya, Hoshiarpur | 2000 |
| | | Sangatpur, Anandpur Sahib road, Ropar | 1000 |
| | | Gara, Anandpur sahib road, Ropar | 1000 |
| 17. | 29.10.2014 | Gandhowaal, Hoshiarpur | 1200 |
| | | Mahilpur, Hoshiarpur | 1000 |
| 18. | 30.10.2014 | Total | 55, 100 |

Table 3. Release of parasitoids in Punjab, during the years 2015-16 and 2016-17.

| S.N. | Date of release | Locality | Number of Parasitoids (Approx.) released |
|------|-----------------|-------------------------------------|--|
| 1. | 16.10.2015 | Bahadurpur, Hoshiarpur | 5000 |
| 2. | 18.10.2015 | Purhiraan, Hoshiarpur | 3000 |
| 3. | 04.11.2015 | Tangra on Amritsar road, Hoshiarpur | 3600 |
| 4. | 06.11.2015 | Shambhu, Rajpura road, Patiala | 4100 |
| 5. | 17.12.2015 | Banga, Phagwara Road, Jalandhar | 2000 |
| 6. | 17.12.2015 | Raikot, Ludhiana | 1500 |
| 7. | 07.04.2016 | Bahadurpur, Hoshiarpur | 500 |
| 8. | 07.04.2016 | Purhiraan, Hoshiarpur | 500 |
| 9. | 23.02.2017 | Bahadurpur, Hoshiarpur | 500 |
| 10. | 24.02.2017 | Village Morinda, Ludhiana | 500 |
| | | Total | 21,200 |

Release of parasitoids in Punjab during the years 2015-17

After two years (2013 and 2014) release of parasitoids at 47 sites in different districts of Punjab, suffering from gall wasp infestation; results of biological control of gall wasp were spectacular and the intensity of gall infestation was reduced systematically. At most of the sites, including experimental release site (Bhunga) attack of gall wasp was reduced. Presence of gall wasp was so less and the gall infestation came down at below economic threshold level, not showing any threat of gall formation. Most of the nurseries and VMG's had become free from gall infestation. Hence, at most of the sites, further release of parasitoids was not required. During 2015-16 and 2016-17, only those sites were taken for release of parasitoids, where the spectacular availability of fresh galls was noticed. Even the galls on these sites were very less and only inoculative release was followed with the release of few parasitoids. Thus the limited release was carried out during these years. Details are given below (Table 3).

Observations and assessment of impact of parasitoids release on gall wasp infestation and galls intensity in different districts of Punjab

Observations were taken during October-November, 2015 and May-July, 2017 on Eucalyptus galls infestation and galls intensity in different districts of Punjab. First detailed observation was taken for an overall field conditions on Eucalyptus gall wasp spreading in different districts. It was aiming at to know whether more intensive releases are required or some new areas should be taken for the release of parasitoids. Galls intensity was increasing or decreasing over all or in limited areas. It would also be reflected whether the released parasitoids were establishing, multiplying and spreading in other areas for having a check and balance on the population of gall forming wasp, *L. invasa*. Five Eucalyptus plantations were taken for observations randomly, in each district. From each plantation, ten plants, selected randomly and one twig per plant was taken for observation. Total number of leaves per twig and leaves with galls per twig were counted. Data of 50 twigs (Total number of leaves and total number of gall infested leaves) were pooled and percentage of

gall infestation was calculated. Data (Table 4) and graph (Figure 6) of the gall intensity in different districts of Punjab are given below:

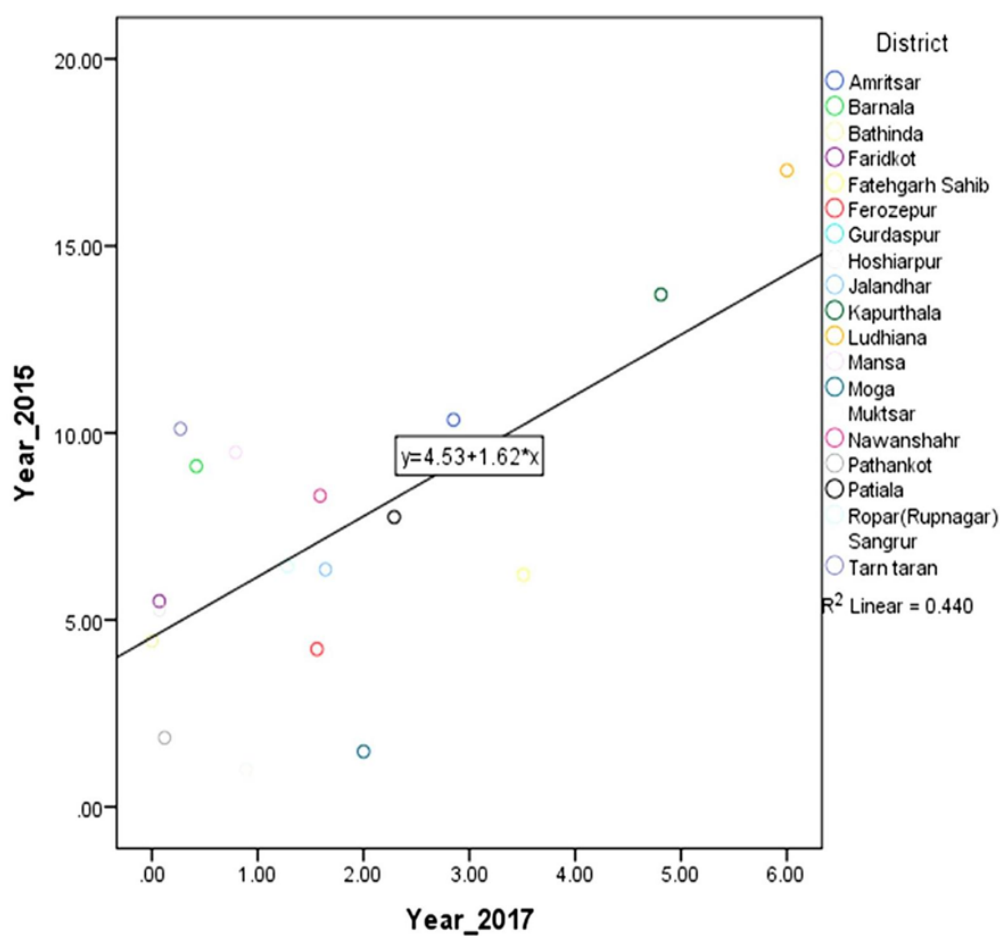
During 2013, as a whole 57,800 parasitoids were released at 12 sites, mostly belonging to districts Hoshiarpur, Gurdaspur and Pathankot; where the gall infestation was severe. District Hoshiarpur was the prime target for the release of parasitoids, as there are several Eucalyptus nurseries, growing clonal material, seedlings and selling the Eucalyptus plants. Eucalyptus VMG's are also there in Hoshiarpur. Hence the severe gall infestation in Hoshiarpur district and adjoining areas was a real threat for Eucalyptus growers, farmers and Nursery Managers. It was a matter of concern also for State Forest Department to control / manage this gall wasp attack. Hence during 2013, focus of parasitoids release was at Hoshiarpur and adjoining Eucalyptus growing areas.

In the second year of release of parasitoids, during 2014, as whole 55,100 parasitoids were released, covering over all 47 release sites including 35 new sites for parasitoids release and thus the parasitoids release was widely spread over 47 sites in 8 districts of Punjab: Hoshiarpur, Pathankot, Gurdaspur, Jalandhar, Kapurthala, Ludhiana, Rupnagar and Patiala. By the release of parasitoids in 2013 and 2014, wide range of Eucalyptus growing areas of Punjab were covered where the gall infestation was observed severe. Thereafter observations were taken on the impact assessment of parasitoids release.

First observation (October-November, 2015) clearly revealed (Figure 6) that the gall intensity was over all decreased after the release of parasitoids. In most of the districts, gall intensity was recorded below 10%, which is a significant observation in the field of biological control of gall wasp, *L. invasa*. In fact Biological control is not for controlling or eradication of a pest species. It is the Management of population of insect pest, below the economic threshold level of injury/ damage. Hence the population of insect pest will be available in nature but it will be checked and balanced by the parasitoids (Bio-control agents). Only in Ludhiana, Kapurthala, Amritsar and Tarn Taran, galls intensity was more than 10%, but in some of these districts population was further decreased and intensity of galls was reduced subsequently.

Table 4. Observations on galls intensity in different districts of Punjab.

| Districts of Punjab | Oct-Nov, 2015 (%) | May-July, 2017 (%) |
|---------------------|-------------------|--------------------|
| Pathankot | 1.85 | 0.12 |
| Muktsar | 1.00 | 0.89 |
| Moga | 1.48 | 2.00 |
| Ferozepur | 4.22 | 1.56 |
| Bathinda | 4.44 | 0.00 |
| Hoshiarpur | 5.27 | 0.07 |
| Faridkot | 5.50 | 0.07 |
| Fatehgarh Sahib | 6.21 | 3.51 |
| Jalandhar | 6.35 | 1.64 |
| Ropar (Rupnagar) | 6.43 | 1.28 |
| Patiala | 7.75 | 2.29 |
| Nawanshahr | 8.32 | 1.59 |
| Sangrur | 8.78 | 1.82 |
| Barnala | 9.11 | 0.42 |
| Mansa | 9.48 | 0.79 |
| Gurdaspur | 9.67 | 2.83 |
| Tarn taran | 10.11 | 0.27 |
| Amritsar | 10.35 | 2.85 |
| Kapurthala | 13.70 | 4.81 |
| Ludhiana | 17.02 | 6.00 |

**Figure 6.** Gall intensity (%) observation in districts of Punjab.

Afterwards, the gall wasp infestation was very less and rarely observed in limited pockets which were targeted during the release of parasitoids in 2015-16 and 2016-17. During this period, as whole 21,200 parasitoids were released at selected sites, including 5 new sites, covering the gall infested sites of three districts: Jalandhar, Hoshiarpur and Ludhiana. Parasitoids release at other places/ sites of Eucalyptus growing areas was not required, as in most of the Eucalyptus nurseries, VMG's and Eucalyptus plantations, gall intensity was decreased as much as Zero or below 10%. Over all 1,34,100 parasitoids were released at 52 sites, mainly covering 8 districts, where the gall infestation was spectacularly high and damaging Eucalyptus nurseries, plantations and other Eucalyptus plants material like VMG's etc.

Finally, second observation was taken on gall intensity in different districts of Punjab in May to July, 2017. Data of second observation clearly revealed that by the July 2017, gall wasp infestation was completely under check and balance. At all sites of observations, galls intensity was recorded below 10%. In most of the cases it was less than 1% and in few cases, galls intensity was more than 1% but it was below 5%, except in one observation i. e. 6% at Ludhiana (Table 4). In case of gall intensity is less than 10 % there is no need of fresh release of parasitoids. Gall forming insect in its biological control is not eradicated from the site and from its acquired Ecological Niche. Only its population is maintained below the economic threshold level of injury/ damage. Therefore the *L. invasa* is available there in Punjab. It will appear time and again, but soon will be checked by the biological control agents, available there, which may be the released parasitoids or some local natural enemies or some other biotic or abiotic factors. There is no need of further release of parasitoids until or unless an outbreak of gall forming insect is observed, in future.

Thus, the gall wasp, *L. invasa* which was posing a serious threat to the Eucalyptus growers, Nursery Managers, and farmers; by developing the severe galls in nurseries and plantations and had become a challenge for its control, where the chemical pesticides were failed to give its control; even after their repeated fortnightly applications. Finally biological control was applied by releasing the larval parasitoid *M. viggianii* and *Q. mendeli*, predominantly *M. viggianii* as a whole 1, 34, 100 parasitoids were released at 52 sites of eight districts of Punjab and the serious galls infestation was brought under check and balance. This is a successful biological control of Eucalyptus gall forming insect *L. invasa*.

Statistical analysis of infestation intensity

For Normality testing, Kolmogorov-Smirnov and Shapiro-Wilk test were conducted with the null hypothesis as "Infestation intensity is not normal". The probability significance for both the years (Oct-Nov, 2015 and May-Jul, 2017) was more than 0.05. Thus, the null hypothesis was rejected and concluded that the data is normal. Further, to test, if there is any effect of parasitoid on infestation intensity, a paired t-test was applied. The null hypothesis (H_0) was "There is no difference in infestation inten-

sity before and after the parasitoid release". The mean infestation intensity ($M=5.61$, $SD=3.14$, $N=20$) was significantly greater than zero, $t=7.98$, two-tail $p=0.0000017$ at 95% confidence interval about mean infestation intensity. Thus, rejecting the null hypothesis (H_0), it can be concluded that the parasitoid release has a positive effect in controlling the gall formation in *Eucalyptus* spp. regardless of districts.

The Blue Gum Chalcid, *Leptocybe invasa* was first recorded in Middle East in 2000 and had spread to most Mediterranean countries, detected in Uganda (2002), Kenya (2002) and Tanzania in (2005) and was reported to spread fast. For knowing the status of gall insect infestations in *Eucalyptus* survey was conducted during December, 2010 by the entomologists of FRI Dehradun in the states: Haryana, Punjab, Uttarakhand and Uttar Pradesh (Kalia et al., 2010) and also survey of plantations and nurseries of Haryana was carried out in August 2011 (Karunaratne et al., 2010; Yousuf and Chauhan, 2011) surveyed Eucalyptus nurseries and plantations covering five Agro-climatic zones of Tamil nadu. Out of the nineteen clones planted, clones like C10, C7, C271, T61 and C 283, were infested with gall insect, *Leptocybe invasa*, ranging from 80-100%. Clone C283 and C271 were found to be most susceptible clones. Clones C274, C226 and IFGTB clones were found free from gall insect. Ramanagouda and Vastrad (2015) reported native parasitoids *Megastigmus dharwadicus* and *Aprostocetus gala* for biological control of eucalyptus gall wasp. They also multiplied two native parasitoids in green house and those were released in a severely affected *Eucalyptus* plantation spread over 1000 ha. A total of 14,000 heavily parasitized galled seedlings, 1400 *M. dharwadicus* and 300 *A. gala* were distributed over a period of six months. Post release evaluation indicated the successful control of the pest. For biological control of gall wasp, the insect galled twigs of eucalyptus were brought from Israel on 19th of November 2008, at National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India. Out of the two consignments of the parasitoid *Quadrastichus*, *Megastigmus* and *Selitrichodes* (male) were emerged (Shylesha, 2012).

Eucalyptus gall infestation was severe on seedlings and saplings in nurseries and also in most of the plantations in Punjab, during (2012). At, Satyal nursery, Hoshiarpur different clones in VMG were infested with galls (Yousuf et al., 2017). Severity of gall wasp attack in Punjab was a serious threat to Eucalyptus and it was a challenging task to control the gall wasp. If the problem would have been unattended, it could inflict the heavy losses to Eucalyptus nurseries, plantations and was imposing a threat of heavy economic losses. Keeping in view all possibilities of implementation of biological control of Eucalyptus gall wasp and following the protocol of National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru with some improvements for controlling the Eucalyptus gall wasp in Punjab, "biological control of Eucalyptus gall wasp, *Leptocybe invasa*" was carried out from August, 2012 to July, 2017. This is a successful biological control of *Eucalyptus* gall forming insect, *Leptocybe invasa*.

Conclusion

During the project period from August 2012 to July 2017, culture of biological control agents of *L. invasa*, larval parasitoid, *M. viggianii* and *Q. mendeli* was brought from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, it was mass multiplied at FRI Dehra Dun and Satyal Nursery, Hoshiarpur for Biological control of gall wasp. After mass multiplication, approximately 1, 34, 100 parasitoids of *M. viggianii* and *Q. mendeli* predominant population of *M. viggianii* were released during 2013-2017. Finally, latest survey (May-July, 2017) revealed that gall infestation in most of the districts of Punjab has systematically gone down up to nil, at maximum *Eucalyptus* growing areas of Punjab. Thus, *Eucalyptus* gall forming insect *L. invasa* has been managed successfully by applying biological control through releasing the parasitoids, in Punjab.

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